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Source of environmental data and warming tolerance estimation in six species of North American larval anurans

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ABSTRACT

The current global warming scenario has led to a renewed interest in determining which species are more vulnerable to climate change. Hence, it is important to understand which factors can affect estimates of species vulnerability. We determined the critical thermal maxima (CT_{max}) for six species of North American anuran larvae and measured the environmental temperatures to which they are exposed during their aquatic stage to estimate their warming tolerance (WT; difference between the critical thermal maximum and the macro- and microhabitat maximum environmental temperatures). Our results indicate that these species exhibited CT_{max} values (37.8–41.7 °C) that were similar to other temperate species and positively correlated only with environmental temperatures measured at the microclimate scale. This indicates that microclimatic variables are better predictors of CT_{max} variation than macroclimate data. Moreover, most of the CT_{max} variation found was associated with higher taxonomic levels, indicating that related species may show similar CT_{max} values due to phylogenetic inertia. Studied species also exhibited high values of WT (10.3–22.6 °C), similar to temperate amphibian species from other bioregions. This indicates that there is a considerable gap between the species' critical thermal maximum and maximum environmental temperature, whether using datalogger (microclimate) or WorldClim (macroclimate) environmental data. However, WT estimates based on datalogger data were not related to those based on macroclimate environmental data. Finally, variation associated with the type of environmental data used (macro- vs. microclimate) had a profound influence on WT estimates. Hence, our perception of which species are more vulnerable to global warming changes may depend on the scale of the climate data used.

1. Introduction

The current IPCC report (2013) predicts that Earth's mean temperature will continue to increase in the coming decades and that these thermal changes will coincide with altered global precipitation patterns and an increase of extreme climatic events (Diffenbaugh and Ashfaq, 2010; Schär et al., 2004). This scenario of climate change has led to an increased interest in how these changes may affect species' phenologies, geographic distributions, and survival, especially for endothermic and ectothermic vertebrates (Chen et al., 2009; Foden et al., 2013; Pacifici et al., 2017; Parmesan, 2006).

Since the physiology, development, and behavior of ectotherms are strongly affected by temperature, most of their physiological processes change rapidly over a range of body temperatures and this relationship defines the thermal performance curve (TPC; Huey and Stevenson, 1979). Thermal performance curves are limited by a critical thermal minimum (CT_{min}) on their lower ends and by a critical thermal maximum (CT_{max}) on their upper ends; temperatures between CT_{min} and CT_{max} define the organism's thermal tolerance range (Huey and Kingsolver, 1989; Huey and Stevenson, 1979; Katzenberger et al., 2012). Experiencing temperatures outside these thermal boundaries often causes death fairly quick; however, exposure to temperatures close to the thermal limits may also result in impaired physiological function (Hillman et al., 2009). Using these thermal performance curves as species traits and making the assumption that ectotherm's physiology is heavily dependent on environmental temperatures, Deutsch et al. (2008) provided two metrics to quantitatively assess the impact of global warming on ectotherms: warming tolerance (WT; the

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difference between the organism's critical thermal maximum and the environmental temperature) and thermal safety margin (TSM; the difference between the organism's optimum temperature and the environmental temperature). Estimating WT and TSM allows for an understanding of the environmental temperature that will cause a given species to start experiencing impaired performance and how fast performance will decline until the species reaches a thermal physiology limit. In turn, these estimates provide insight into how changes in the environmental temperature may affect those species.

The current scenario of climate change has prompted an interest in relating the thermal physiology with impacts of global warming and potential adaptive responses in amphibian species (e.g. Duarte et al., 2012; Gutiérrez-Pesquera et al., 2016; Simon et al., 2015; Thurman and Garcia, 2017). Hence, the WT and TSM metrics have been used in several studies that either compiled available data or produced new physiological information for several taxa (Deutsch et al., 2008; Duarte et al., 2012; Sunday et al., 2014, 2011). To estimate WT and TSM, these studies used either macroclimate (Deutsch et al., 2008; Sunday et al., 2014, 2011) or microclimate data (Duarte et al., 2012). The recent works of Duarte et al. (2012) and Gutiérrez-Pesquera et al. (2016) comparing communities of larval amphibians from biomes with contrasting thermal regimes (tropical, subtropical and temperate), showed that CT_{max} variation has a phylogenetic signal and is positively correlated with maximum environmental temperature. In addition, temperate species could be less vulnerable to global warming than their tropical counterparts (Duarte et al., 2012; Gutiérrez-Pesquera et al., 2016; Sunday et al., 2014), as predicted in recent works (Deutsch et al., 2008; Tewksbury et al., 2008).

There is a general understanding on how thermal physiology traits can be affected by methodological details as well as several biotic and abiotic factors (e.g. Camacho and Rusch, 2017), which can subsequently influence WT estimates. In CT_{max} estimation, using loss-of-righting response as an endpoint can provide estimates up to 4.1 °C lower than the onset of spasms as an endpoint in turtles (Hutchison et al., 1966). Moreover, as an endpoint, complete immobility is physiologically much closer to the onset of spasms than to the loss-of-righting response (see Lutterschmidt and Hutchison, 1997a, 1997b). Acclimation to higher temperatures usually provide higher estimates of CT_{max} and vice-versa (Brattstrom, 1968; Navas et al., 2008; Simon et al., 2015). In addition, CT_{max} estimates can be affected by photoperiod (Brattstrom, 1968; Hutchison and Kosh, 1965; Mahoney and Hutchison, 1969), the heating rate used (i.e. ramping; Chown et al., 2009; Mitchell and Hoffmann, 2010; Rezende et al., 2011; Ribeiro et al., 2012; Terblanche et al., 2007), and ontogeny (Floyd, 1983; Sherman, 1980).

Another important factor that may affect estimates of WT is the environmental temperature data that is used (micro- vs. macroclimate). Macroclimate environmental data, such as WorldClim (Hijmans et al., 2005), is usually based on air temperature information collected over several years (even decades), often by weather stations (or similar apparatus), and extrapolated over large areas. Microclimate environmental data is usually a better proxy of the conditions experienced by the organisms (see Pincebourde and Suppo, 2016), but it is much harder to collect over extended periods of time and more difficult to extrapolate for the species distribution. Some studies that used microclimate data (e.g. Duarte et al., 2012; Gutiérrez-Pesquera et al., 2016) confirmed the predictions made using macroclimate data (Deutsch et al., 2008; Tewksbury et al., 2008), even though species occurring in the same geographic region may present different levels of vulnerability to increased environmental temperatures. However, Logan et al. (2013) suggested that the vulnerability of several tropical ectotherms at a regional scale may have been overestimated by these large-scale predictions based on low-resolution temperature data. Hence, if using macro- and microclimate environmental data results in WT estimates with significant differences in magnitude, the perspective of which species are more vulnerable to global warming may depend on the database used. This would also affect the application of these metrics in other instances, such as the decision-making process to determine priority species/areas for conservation efforts.

The current study aims to assess how the perception of vulnerability to global warming may be influenced by the source of environmental data, while also contributing with additional information regarding the upper thermal tolerances of North American amphibian species. Critical thermal maxima and WT (Deutsch et al., 2008) were estimated to determine how close environmental temperatures are to each species' thermal limits, considering both macro- and microclimate environmental information. Based on previous findings, we expect part of the variation in species' critical thermal maxima to be associated with the phylogenetic relationship among studied species. In addition, it has been suggested that upper tolerance limits in ectotherms are correlated with maximum microhabitat temperatures (Araújo et al., 2013; Bernal and Lynch, 2013; Compton et al., 2007; Duarte et al., 2012; Stillman and Somero, 2000), albeit less correlated than in cold tolerances (Araújo et al., 2013; Gutiérrez-Pesquera et al., 2016). Considering that the latitudinal gradient of seasonality is more associated with variation in minimum temperatures than with maximum temperatures (Ghalambor et al., 2006), we also expect to determine which elements of the thermal profile (maximum, mean, and minimum temperatures and diel temperature fluctuation) can constitute good predictors of CT_{max} (for both micro- and macroenvironmental data). Furthermore, studied species may present high WT, as seen in other temperate species (Duarte et al., 2012; Gutiérrez-Pesquera et al., 2016).

2. Methods

2.1. Quantifying critical thermal tolerance

In spring 2011, we collected newly fertilized eggs from six amphibian species from natural ponds and wetlands (Table 1) and brought them to the University of Pittsburgh's Pymatuning Laboratory of Ecology, in northwest Pennsylvania, USA. Species not from Pennsylvania were mailed overnight (as embryos) from their collection sites to the Pymatuning Laboratory of Ecology, to be reared alongside the other species, in a common garden setting. Studied species encompass representatives of bufonids, hylids and ranids with broad distribution. Although *Rana cascadae* presents a more restricted distribution, it was included to represent species generally found in mountain ranges (in this case, from western North America).

Table 1
Location and altitude of collection site and number of tadpoles collected (n) per species of six amphibians from the United States of America.

Species (n)	Genus	Family	Location	Latitude	Longitude	Altitude (m)
Anaxyrus americanus (15)	*Anaxyrus*	Bufonidae	Crawford County, PA[¥]	41°41′16.33″N	80°25′33.89″W	333
Hyla versicolor (15)	*Hyla*	Hylidae	Crawford County, PA	41°41′27.96″N	80°29′57.07″W	317
Pseudacris crucifer (15)	*Pseudacris*	Hylidae	Crawford County, PA	41°34′08.60″N	80°27′09.03″W	371
Rana cascadae (15)	*Rana*	Ranidae	Linn County, OR	44°28′50.31″N	121°59′39.68″W	1139
Rana luteiventris (15)	*Rana*	Ranidae	Latah County, ID	46°44′07.65″N	116°55′16.02″W	863
Lithobates sphenocephalus (14)	*Lithobates*	Ranidae	Craven County, NC	34°54′38.00″N	76°59′15.00″W	10

[¥] PA, Pennsylvania; OR, Oregon; ID, Idaho; NC, North Carolina.

Amphibian eggs were placed in outdoor pools that were filled with well water, keeping each species separate. After the eggs hatched, tadpoles were fed rabbit chow ad libitum until their thermal physiology was assessed. After tadpoles reached Gosner stage 25 and a body mass above 100 mg (except for *Anaxyrus americanus*, formerly *Bufo americanus,* whose tadpoles are smaller), we brought a set of tadpoles from each species into the laboratory where they were kept at an acclimation temperature of 20 °C (approximately the mean temperature experienced while in the outdoor pools). In the lab, we raised them under a 12 L:12D photoperiod and continued to feed them rabbit pellets ad libitum. After 4–5 d of acclimation, we tested the tadpoles for CT_{max} (Brattstrom, 1968; Hutchison, 1961). Since tadpoles close to metamorphic climax can exhibit a significant decline in thermal tolerance (Sherman, 1980), we only tested larvae below Gosner stage 38 (Gosner, 1960).

To obtain estimates of the upper critical thermal tolerances (CT_{max}), we used a modified version of Hutchison's dynamic method (Lutterschmidt and Hutchison, 1997b) by exposing tadpoles to a constant heating rate of 0.05 °C min^{-1} (i.e. 3 °C h^{-1}). This rate has been used in previous studies (Katzenberger et al., 2014) because it simulates a rate of temperature increase that has been observed in ponds (Duarte et al., 2012; Terblanche et al., 2011; current study). The endpoint of the critical thermal tolerance experiments was defined as when the tadpole became immobile, after gently prodding them with a stick. Once CT_{max} was reached, tadpoles were transferred to cooler water (± 20 °C) to allow complete recovery. After the tadpoles recovered, we weighed them. We tested 15 tadpoles per species and only tadpoles that fully recovered were included in subsequent analysis. All experiments were approved by the University of Pittsburgh's Institutional Animal Care and Use Committee (Protocol #12050451).

2.2. Quantifying environmental temperatures

We took two approaches to obtain environmental temperatures to which these species are exposed during their breeding season. First, we assessed microenvironmental pond temperatures by placing HOBO Pendant® temperature dataloggers in each collection site (at the deepest part of the pond) and recorded water temperature every 5–15 min, during each species' breeding season (Table 2). Second, we mapped and extracted macroenvironmental air temperature information from a spatially interpolated monthly climate dataset (with 1-km^2 spatial resolution) for global land areas (WorldClim; Fick and Hijmans, 2017) for each collection site, using QGIS software. In both cases, we determined for the time period considered: maximum temperature recorded (T_{max}); mean temperature (T_{mean}), minimum temperature recorded (T_{min}), and annual and average diel temperature fluctuation (DTF) for macro and microenvironmental estimates, respectively (Table 2). This allowed us to determine if CT_{max} was related to any of the thermal profile metrics. For microenvironmental data, DTF was determined for the time period considered in Table 2 (DTF_{avg}, average diel temperature fluctuation), while for macroenvironmental data DTF was determined on an annual basis, as provided in WorldClim BIO2 (DTF_{annual}, annual diel temperature fluctuation; Table 2).

To assess each species' vulnerability to increasing environmental temperatures, we estimated WT for each species as the difference between the species' upper critical thermal limit (CT_{max}) and its current maximum environmental temperature (T_{max}). Since we have both micro (datalogger; maximum water temperature recorded at the deepest part of the pond) and macroenvironmental information (WorldClim; maximum air temperature recorded), we determined two estimates of WT for each species (WT_d and WT_w, respectively).

2.3. Statistical analysis

We performed an ANCOVA analysis, using species as a categorical factor and mass as a covariate, to determine if species differed in their upper critical thermal limits. We followed this analysis with a Tukey HSD post-hoc test to see which species differed from each other.

Incorporating phylogenetic information is important when performing statistical analyses in a multiple-species dataset (see Felsenstein, 1985; Garland et al., 1992). However, the number of species included in this study is below the > 20 species threshold recommended for PGLS analysis (see Blomberg et al., 2003). Hence, we conducted a linear mixed model fit using maximum likelihood, where the taxonomic levels of genus and family were included as nested random effects to account for the non-independence of related species (see Seebacher et al., 2015). Furthermore, species and mass were included in the model as fixed factors. This analysis allowed to determine if the variation associated with higher taxonomic levels is responsible for differences in CT_{max} at species level. In addition, we followed Pyron and Wiens (2011) to extract a phylogenetic tree for the sampled species, keeping both topology and estimated branch lengths. These branches were color-coded to indicate how CT_{max} varied within the phylogenetic tree (Fig. 1).

We used general linear models (GLMs) to determine the relation between the critical thermal maximum and the environmental temperature data (T_{max}, T_{avg}, T_{min} and DTF). This would allow us to evaluate which traits of the thermal regime (maximum, mean or minimum temperatures and/or diel temperature fluctuations) were more influential in determining the critical thermal maximum for the studied species. We conducted ANOVA analysis, followed by Tukey HSD, to see if there were interspecific differences in between WT estimate based on either microenvironmental or macroenvironmental data. To determine if WT estimates differed depending on the environmental data used, we performed a two-sample *t*-test for each species. We also compared WT estimates using Pearson product-moment correlation to determine if, despite using different environmental data, these estimates could provide a similar assessment of species vulnerability to global warming.

Table 2
Estimates of environmental T_{max} (maximum temperature recorded), T_{mean} (mean temperature), T_{min} (minimum temperature recorded) and, DTF_{annual} and DTF_{avg} (annual and average diel temperature fluctuation, respectively) for six amphibian species, obtained from WorldClim database (Fick and Hijmans, 2017) and locally placed dataloggers. Time period considers the months that were sampled from both datalogger and WorldClim data.

Species	WorlClim data				Datalogger data				Time period considered
	T_{max}	T_{mean}	T_{min}	DTF_{annual}[€]	T_{max}	T_{mean}	T_{min}	DTF_{avg}	
Anaxyrus americanus	24.6	16.1	7.6	10.4	25.7	19.2	9.7	3.7	May-June
Hyla versicolor	25.3	19.8	12.5	10.4	29.5	23	15.4	5.6	June-July
Pseudacris crucifer	25.3	15.1	1.2	10.7	26.1	16.3	5.6	4.0	April-July
Rana cascadae[¥]	19.2	5.1	− 7.6	12.2	15.3	9.8	4	1.7	February-June
Rana luteiventris[¥]	22.7	7.6	− 7.0	11.7	15.3	9.8	4	1.7	February-June
Lithobates sphenocephalus[¥]	26.3	1863	10.7	12.2	25.1	20.9	15	2.1	April-May

[€] DTF_{annual} was determined on an annual basis (as provided in WorldClim BIO2), while DTF_{avg} was determined for the time period shown.
[¥] For these species, datalogger environmental information for the collection site was unavailable so a thermal profile from a similar pond in the species' distribution was used instead.

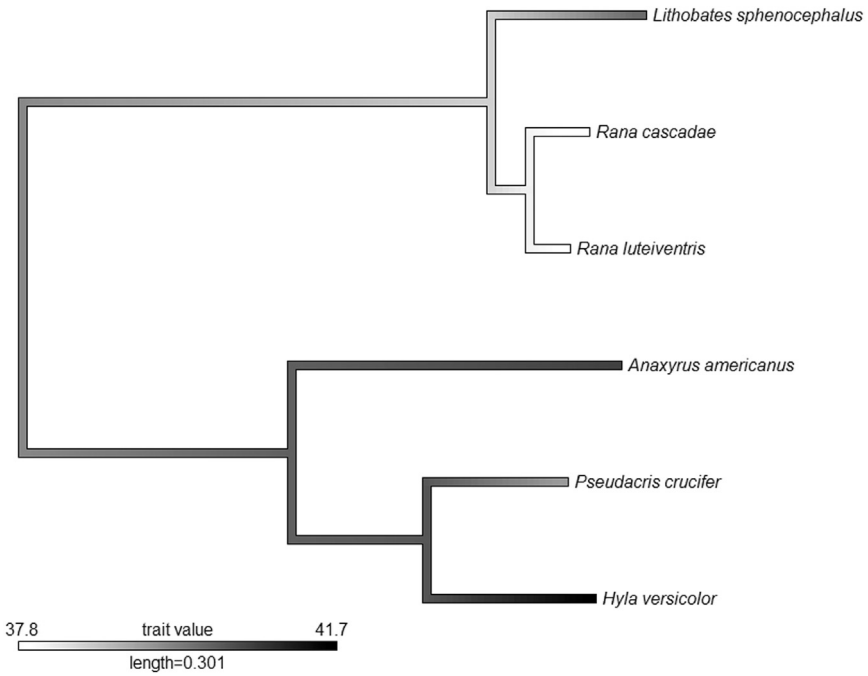

Fig. 1. Phylogenetic tree of six amphibian species based on Pyron and Wiens (2011). Branches are color coded according to each species' critical thermal maximum (in °C), from white (lower) to black (higher).

All analyses were performed in R (R Development core team 2014) and conducted at the significance level of $\alpha = 0.05$.

3. Results

The ANCOVA analysis revealed that species differed in their critical thermal maximum ($F_{(5,77)} = 145.4$, $p < 0.001$; Table 3). However, neither mass ($F_{(1,77)} = 0.7$, $p = 0.42$) nor the species-by-mass interaction ($F_{(5,77)} = 0.5$, $p = 0.79$) had any effect on critical thermal maximum. Furthermore, in the post-hoc tests, all species' comparisons differed in their CT_{max} (all Tukey HSD $p < 0.05$), except for *Rana luteiventris* versus *Rana cascadae* (Tukey HSD $p = 0.99$). However, when accounting for higher taxonomic levels (linear mixed model), neither species ($\beta = 0.01 \pm 0.16$, $t = 0.05$, $p = 0.96$) nor mass ($\beta = 0.32 \pm 0.47$, $t = 0.68$, $p = 0.49$) were significant factors determining CT_{max}. This indicates that the variation responsible for species differences in CT_{max} can occur at higher taxonomic levels (particularly genus; Table 4), as the two species with the lowest CT_{max} belonged to genus Rana (*R. luteiventris* and *R. cascadae*; Table 3, Fig. 1).

Regarding the relation between critical thermal maximum and the environmental temperature metrics, CT_{max} increased with maximum, mean and minimum environmental temperatures estimated using dataloggers (Table 5). However, this relation was only observed for mean and minimum environmental temperatures when using WorldClim data. CT_{max} also increased with average diel temperature fluctuation (DTF_{avg}, datalogger) but was not related to annual diel temperature fluctuation (DTF_{annual}, WorldClim).

Table 4
Linear mixed model fit using maximum likelihood to determine the relation between critical thermal maximum and the fixed factors species and mass. Taxonomic levels genus and family were included as nested random effects to account for the non-independence of related species.

Fixed parameters	estimate	SE	t	*p*
intercept	39.84	0.82	48.76	< 0.001
species	0.01	0.16	0.05	0.964
mass	0.32	0.47	0.68	0.494
Random effects	**variance**	**SD**		
Family:genus	0.258	0.51		
genus	1.404	1.18		
Residual	0.235	0.48		

All species presented high values of WT (Table 3, Fig. 2), regardless of environmental data used. Considering the microenvironmental data, WT estimates differed among all species ($F_{(5, 83)} = 1340.0$, $p < 0.001$; see Supplementary information I), except for *A. americanus*-L. *sphenocephalus* (Tukey HSD $p = 0.996$) and *R. cascadae-R. luteiventris* (Tukey HSD $p = 0.991$). Regarding macroenvironmental data, WT results were similar ($F_{(5, 83)} = 194.5$, $p < 0.001$), but the species that did not differ were *A. americanus-H. versicolor* (Tukey HSD $p = 0.837$) and *P. crucifer*-L. *sphenocephalus* (Tukey HSD $p = 0.507$). WT estimates for each

Table 3
Critical thermal maximum (CT_{max} in °C ± SE), mass (in mg ± SE), Gosner stage (average ± SE), Warming Tolerance (in °C), estimated using both datalogger (WT_d) and WorldClim (WT_w) data, and the difference between WT estimates (diff), for six species of North America anuran larvae.

Species	CT_{max}	Mass (mg)	Gosner	WT_d	WT_w	diff
Anaxyrus americanus	40.7 ± 0.1	80.1 ± 10.2	31.6 ± 0.6	15.0 ± 0.1	16.1 ± 0.1	− 1.1*
Hyla versicolor	41.7 ± 0.1	323.0 ± 22.7	34.5 ± 0.5	12.2 ± 0.1	16.4 ± 0.1	− 4.2*
Pseudacris crucifer	39.4 ± 0.1	137.9 ± 9.1	34.3 ± 0.8	13.3 ± 0.1	14.1 ± 0.1	− 0.8*
Rana cascadae	37.8 ± 0.1	385.9 ± 36.1	30.9 ± 0.3	22.5 ± 0.1	18.6 ± 0.1	3.9*
Rana luteiventris	37.9 ± 0.2	333.9 ± 23.7	29.1 ± 0.5	22.6 ± 0.2	15.2 ± 0.2	7.4*
Lithobates sphenocephalus	40.1 ± 0.1	234.4 ± 53.0	26.0 ± 0.3	15.0 ± 0.1	13.8 ± 0.1	1.2*

Significant differences between WT estimates are marked with *.

Table 5
Relation between the critical thermal maximum and the environmental temperature data (T_{max}, T_{mean}, T_{min} and DTF), using general linear models. Coefficients are presented as β ± SE.

	datalogger	WordClim
T_{max} [¥]	β = 0.24 ± 0.04, t = 5.51, p = 0.005	β = 0.45 ± 0.20, t = 2.28, p = 0.085
T_{mean}	β = 0.27 ± 0.03, t = 7.7, p = 0.002	β = 0.24 ± 0.05, t = 5.21, p = 0.006
T_{min}	β = 0.25 ± 0.07, t = 3.45, p = 0.026	β = 0.17 ± 0.03, t = 6.65, p = 0.003
DTF_{avg}/DTF_{annual}	β = 0.82 ± 0.27, t = 2.99, p = 0.04	β = −1.22 ± 0.65, t = −1.88, p = 0.132

[¥] T_{max}, maximum environmental temperature; T_{mean}, mean environmental temperature; T_{min}, minimum environmental temperature; DTF_{avg}, average diel temperature fluctuation (datalogger); and DTF_{annual}, annual diel temperature fluctuation.

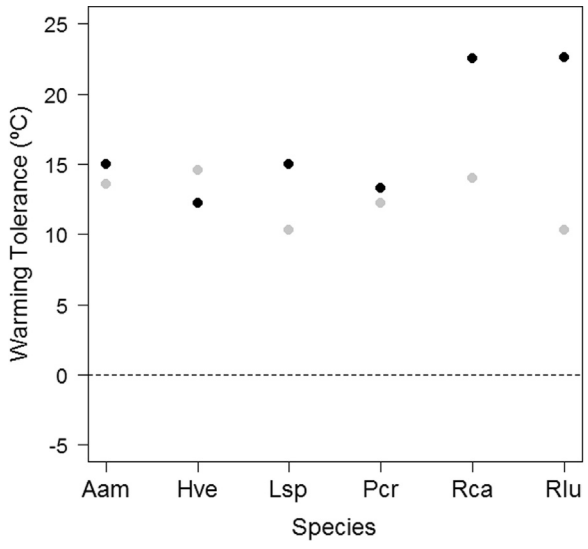

Fig. 2. Estimates of Warming Tolerance (WT) for six amphibian species from North America. WT estimates using datalogger environmental temperatures are shown in black and WT estimates using WorldClim environmental data are shown in grey. Species: Aam, *Anaxyrus americanus*; Hve, *Hyla versicolor*; Pcr, *Pseudacris crucifer*; Rca, *Rana cascadae*; Rlu, *Rana luteiventris*; Lsp, *Lithobates sphenocephalus*.

species differed (all *t*-test $p < 0.001$) depending on the environmental data used, with those calculated from datalogger information (12.2–22.6 °C) being much more variable than those determined using WorldClim data (13.8–18.6 °C). Moreover, WT estimates obtained with datalogger environmental information were not related to those obtained using the WorlClim database (Pearson's r = 0.39, p = 0.443).

4. Discussion

The estimation of thermal physiology limits has gained renewed attention in the current scenario of climate change, and is essential for the assessment of species' vulnerability to increasing environmental temperatures (Deutsch et al., 2008; Duarte et al., 2012; Sunday et al., 2014, 2011). The critical thermal maximum values measured for these six North American species fall within the range (approx. 33–42 °C) previously reported for temperate larval amphibians (Duarte et al., 2012; Gutiérrez-Pesquera et al., 2016; Katzenberger et al., 2014; Navas et al., 2010). For three of our six species, we found reference to their upper thermal limits in the literature, although there were differences in the methodological protocols. As some of our results differed from those previously obtained in other studies, depending on which methodological protocol was used (see Supplementary information II for a detailed discussion), this variation in CT_{max} estimates can also contribute to differences in the assessment of species' WT, in particular when pooling data from different works. In addition, our data also indicates that closely related frog species present similar estimates of CT_{max}. This highlights the importance of considering phylogeny when studying thermal limits, as seen in larger datasets which have detected phylogenetic signal in CT_{max} in both the adult stage (Navas et al., 2008) and in the larval stage (Duarte et al., 2012; Gutiérrez-Pesquera et al., 2016).

Compared to the WT values (between 1.4 and 18.8 °C) estimated for Palearctic amphibian species by Duarte et al. (2012), our six North American species exhibited WT values on the higher end of the range, with some species even surpassing it (WT_d, 12.2–22.6 °C; WT_w, 13.8–18.6 °C). This indicates that current maximum environmental temperatures are generally much lower than their critical thermal maximum at the studied sites, which suggests that these species are relatively safe regarding the direct impact of maximum environmental temperatures (Duarte et al., 2012; Gutiérrez-Pesquera et al., 2016). These results are in line with previous predictions that temperate amphibians (and other ectotherms) were not as vulnerable as their tropical counterparts (Deutsch et al., 2008; Duarte et al., 2012; Gutiérrez-Pesquera et al., 2016; Huey et al., 2009; Sunday et al., 2014; Tewksbury et al., 2008) and could even benefit from increasing environmental temperatures if their optimum temperature is not surpassed (see Tewksbury et al., 2008). However, it is important to mention that some temperate species can be vulnerable to increasing environmental temperatures (Duarte et al., 2012) and that current predictions indicate asymmetrical changes in the thermal regimes, with temperature increasing more in higher latitudes (IPCC, 2013). Furthermore, we cannot discard that other North American amphibians, in particular those with more southern distributions and late-season reproduction, may be exposed to high pond temperatures close to their tadpole upper thermal resistance.

Considering the different sources of environmental data, variation in upper thermal tolerances reflected adaptation to the prevalent microenvironmental (pond) maximum temperatures, as seen in Duarte et al. (2012) and Gutiérrez-Pesquera et al. (2016), but not between CT_{max} and macroenvironmental data. Furthermore, CT_{max} also increased with higher mean and minimum micro- and macroenvironmental temperatures, thus supporting the notion of species' adaptation to their thermal environment. WorldClim provides macroenvironmental data regarding a 30-year period (Fick and Hijmans, 2017), but when extracting data for the relevant months (Table 2), it includes air temperature values below the freezing temperature of ponds, consequently affecting the estimation of mean and minimum temperatures for some of the species. These environmental temperatures would only be biologically significant if the ponds did not completely freeze and/or if tadpoles could sustain being frozen for some period of time (see Navas et al., 2010). This suggests that, for these north-American species, although macroclimate data can produce similar results for some variables, microclimatic data is still a better predictor of the variation in their upper thermal limits than macroclimatic data (see Gutiérrez-Pesquera et al., 2016).

Differences in WT estimates based on micro- and macroclimate information can be associated with the way each database is constructed. The 1-km^2 spatial resolution of macroclimate information (obtained through data extrapolation) gives us the assumption that large areas are

homogeneous, but for tadpoles and other smaller animals at this resolution they are not. Contrarily, microclimate data is able to improve the capture of the spatial heterogeneity that species experience, particularly when measured at a scale compatible with the size and microhabitat of the studied species (Navas et al., 2013), and provide a more detailed description of their operative temperatures (e.g. Logan et al., 2013; Storlie et al., 2014). The quality of microclimate data can be further improved by using dataloggers that resemble the physical properties of the studied organisms (Navas and Araujo, 2000). Furthermore, relevant estimates of thermal environments can also be obtained by statistical downscaling of lower spatial resolution macroclimate data (Storlie et al., 2014).

Although CT_{max} can contribute to differences in WT estimates, most of the variation in WT appears to be associated with environmental temperatures used in WT calculations, in particular when using datalogger information. In our study, a difference of 4 °C between the highest and lowest CT_{max} estimates for the studied species resulted in a 10 °C difference between the highest and lowest WT values (datalogger; Table 3). Similarly, Duarte et al. (2012) found that a 5 °C difference in CT_{max} (35.4–40.0 °C) corresponded to a 17 °C difference in WT (1.4–18.8 °C), also when using microclimate data. WT values do not vary in the same direction for all species when comparing WT_d and WT_w. Furthermore, the magnitude of changes (regardless of direction) is also different amongst species, hindering the assessment of which species are more vulnerable to high environmental temperatures. The most notorious example of this is *Hyla versicolor*, which presented the second highest WT when calculated with WorldClim data and the lowest WT when estimated using datalogger information. In addition, *R. luteiventris* presented a 7.4 °C difference between estimates (Table 3). Depending of the WT estimate used, the perception on how much temperature increase this species could tolerate before suffering impaired physiological functions (while approaching CT_{max}) could vary.

Finally, several studies have demonstrated within-species variation in heat tolerance (Brattstrom, 1970, 1968; Garland Jr. and Adolph, 1991; Gvoždík and Castilla, 2001; Hertz et al., 1979; Huang and Tu, 2008; Hutchison, 1961; Miller and Packard, 1977; Winne and Keck, 2005) and that it could also vary adaptively amongst populations (Richter-Boix et al., 2015; Skelly and Freidenburg, 2000; Wu and Kam, 2005), including adaptation responses to different environmental regimes on the course of several generations (see Skelly et al., 2007). Since different microhabitats vary in thermal traits such as maximum, mean and minimum environmental temperatures and DTF (Richter-Boix et al., 2015), this microenvironmental heterogeneity that species experience, associated with local adaptation of organisms to the thermal regime, will likely result in different WT estimates for different populations. Furthermore, species' behavior and their use of available microhabitats may vary with location and time of year, as environment conditions change (see McCann et al., 2017). Species distribution models that use macroclimate databases, such as WorldClim, often fail to account for this physiological, behavioral and microclimate variation amongst populations. Thus, some species distribution models may be producing a biased perspective on species vulnerability and responses to climate change (e.g. Woodin et al., 2013), in particular for aquatic and/or smaller sized species which explore different microhabitats. Incorporating microclimate data to these models, in addition to accounting for variation associated with methodological factors, would be of great importance to improve their accuracy and predictive capability. Despite being a major challenge and requiring a considerable amount of effort, obtaining relevant microclimate data for each species distribution, and understanding the behavioral responses behind microhabitat use, are fundamental in the assessment of climate change impacts on species.

5. Conclusions

In the current scenario of climate change, it has become increasingly important to understand how close the environment is to each species' physiological limits. Our CT_{max} estimates were within the range of expected values for temperate species but, different methodological protocols hinder comparisons amongst studies. As a result, future studies should consider an extensive review on how each methodological factor affects the estimation of thermal physiology traits and how it varies with species biology. Moreover, researchers should reach agreement methodological protocols that all studies use, so that studies provide comparable estimates. This would help large-scale vulnerability assessments, particularly when pooling data from multiple studies (e.g. Sunday et al., 2014).

Variation associated with the type of environmental data used (macro- vs. microclimate) can have a profound influence on WT estimates, to the point that our perception of species vulnerability to global warming changes depending on the database used. Hence, future larger-scale assessments should not only consider changes in physiological function due to increasing temperature and how it may interact with phenotypic changes caused by other types of environmental variation (Chown et al., 2010; Chown and Terblanche, 2007; Hoffmann, 2010; Katzenberger et al., 2014; Pörtner and Farrell, 2008), but also include microclimate environmental data and species behavior (regarding microhabitat use) to provide a more accurate assessment of vulnerability to global warming.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jtherbio.2018.07.005.

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